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Transmission Electron Microscopy Identification of VPH16 L1 His-Tag Inclusion Bodies in *Escherichia Coli* SHuffle T7

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Cervical cancer is caused by persistent infections of human papillomavirus (HPV), mainly HPV 16. Major capsid protein L1 of HPV can self-assemble into virus-like particles (VLP), which have been used as vaccine candidates [1, 2].

HPV 16 L1 protein can be produced in a fused form (with a His-tag residue) in *Escherichia coli* [3].

The objective of this work was to verify the possible formation of HPV16 L1 His-tag aggregates in *E. coli* SHuffle T7 by means of Transmission Electron Microscopy (TEM).

Three samples of *E. coli* SHuffle T7 were studied: a pETHPV16L1myc-His plasmid sample and two controls (wild strain and pet 28a vector) [4].

E. coli cells were cultured in Terrific Broth medium and induced by the addition of 0.5 mM isopropyl-β-D-1- thiogalactopyranoside (IPTG) during 3 h at 30 ºC. After centrifugation, cell pellets were fixed in 2 % glutaraldehyde in sodium phosphate buffer (0.1 M, pH 7.2), during 12 hours, postfixed in 1 % osmium tetroxide in the same buffer, dehydrated in acetone (30, 50, 70 y 100 %) and embedded in Spurr resin.

Ultrathin sections were obtained and contrasted with uranyl acetate and lead citrate.

Cells were visualized using a Jeol JEM 1011 TEM.

Normal bacterial structure was observed in wild strain and pet 28a vector *E. coli* SHuffle T7 samples (Fig. 1 A, B). However, *E. coli* SHuffle T7 pETHPV16L1myc-His exhibited dense areas throughout the cytoplasm, suggesting the presence of inclusion bodies (Fig. 1 C). The TEM study has confirmed the insoluble production of pETHPV16L1myc-His protein as inclusion bodies, which could be used as a starting material for protein purification to develop a VLP-based HPV vaccine.

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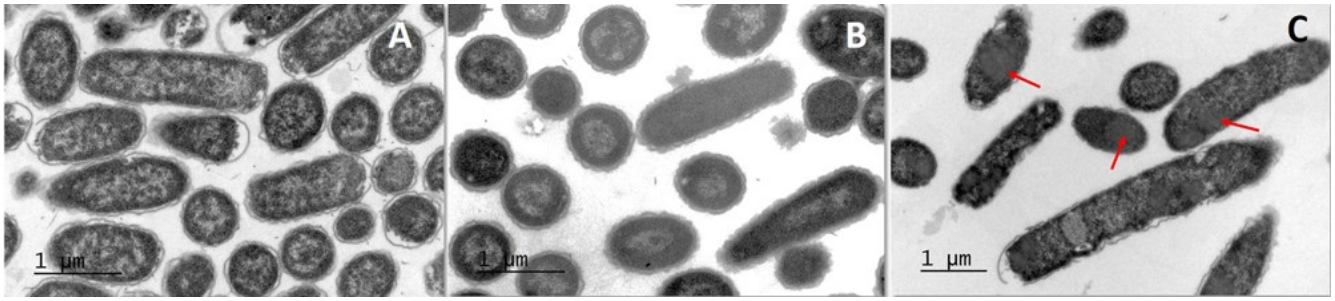

Figure 1: Transmission Electron Microscopy micrographs. A) *E. coli* Shuffle T7, B) *E. coli* SHuffle T7 with pet 28a vector, C) *E. coli* SHuffle T7 pETHPV16L1myc-His. Arrows: inclusion bodies.

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